

# Similarities of Lipid Metabolism in Mammalian and Protozoan Cells: an Evolutionary Hypothesis for the Prevalence of Atheroma

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## INTRODUCTION

It is today widely accepted that cholesterol esters of fatty acids, derived from the diet, are carried in plasma low-density lipoprotein (LDL) and that high levels of plasma cholesterol are associated with the deposition of plaques, consisting mainly of cholesterol ester and crystals of free cholesterol, in the aortic smooth muscle cell (ASMC).

There is a similar association, less widely recognized, between the absorption of chole-

sterol esters of fatty acids and the behavior of certain parasitic protozoa which are found in the blood. This relationship is most clearly seen in *Trypanosoma brucei*, in which cholesterol fatty acid esters enter the trypanosome cell and it is destroyed in a manner strikingly similar to the process with ASMC. Related mechanisms of lipid absorption and sometimes of cytolysis are also found in other pathogenic protozoa, but they are less clearly defined and their similarity to the destruction of the ASMC is less obvious.

The purpose of this review is to compare and

contrast the mechanisms of uptake of cholesterol and fatty acids and their storage and lethal properties for both mammalian and various model protozoan cells; we will also put forward hypotheses to explain the facts. Discussion of the process in the ASMC need only be brief, amounting to a setting of the scene, since the work is generally well known and has been ably described in a number of recent reviews (3, 9, 46). More detail is necessary for the protozoan cells, since the relevant work has not hitherto been reviewed. The fact that there are important gaps in our knowledge of the lipid metabolism of pathogenic protozoa does not seem a reason for postponing such a review; rather, such a review should serve as a means of emphasizing the urgency of filling the gaps.

## CHOLESTEROL ESTER ACCUMULATION

### Aortic Smooth Muscle Cells

All eucaryotic cells require cholesterol and fatty acids for the synthesis of their membranes, and mammalian cells obtain them in two ways: by direct synthesis within the cell (29, 88) and by transport via the plasma from sites of synthesis elsewhere in the body or from the diet. About three-quarters of the plasma cholesterol is carried in the  $\beta$ -lipoprotein, or LDL, and most of the remainder of the  $\alpha$ -lipoprotein, or high-density lipoprotein (HDL); a small fraction occurs in the pre- $\beta$ , or very low-density, lipoprotein (78). The cholesterol esters of fatty acids, which are by themselves insoluble in plasma, are located in the LDL as a nonpolar core surrounded with a polar shell of phospholipids, apoprotein, and unesterified cholesterol, thus ensuring solubilization and transport. Normally the synthesis of cholesterol and its conversion to the ester is regulated in the cell by a homeostatic mechanism based on the amount of LDL cholesterol presented to the outside of the cell (8). Various mechanical and toxic factors have been suggested as means by which the homeostatic mechanism can be deranged, but probably the most important clinical factor in increasing the storage of cholesterol, at least in ASMC, appears to be an increase in the dietary consumption of cholesterol and of saturated fatty acids, which have the specific effect of increasing the amount of cholesterol incorporated in the lipoprotein and therefore available to be taken up from the blood. The result is an enhanced uptake and storage of cholesterol esters, which appear as globules within the cell, giving it the characteristic appearance of a "foam cell" (42). ASMC seem to be particularly at risk, presumably because of the structural role that they play in the body and because they cannot easily be re-

placed; they are transformed into the atheromatous plaques of the arterial wall which Virchow (91) recognized as consisting mainly of lipid. Besides humans, a number of different species of animals also develop atheroma under the influence of a high-cholesterol diet; an experimental model has been developed in rabbits, which, when fed on a diet high in cholesterol, develop, as do other species, typical foam cells in the aorta. In this model (63), large globules of cholesterol ester accumulate and are associated with the release of hydrolytic enzymes, including those usually associated with the activation of lysosomes, and these enzymes gradually destroy the cell, leaving behind a deposit of cholesterol and cholesterol ester.

Although LDL is recognized as the main carrier of cholesterol, attention has recently been focused on the action of HDL, which may reverse the process of cholesterol accumulation in ASMC (11, 66). Experiments with fibroblasts cultured in vitro have demonstrated the existence of receptors for LDL to which HDL may also become attached, and it is thought that this attachment of HDL may be responsible for removal of excess cholesterol from the cell (53). An alternative hypothesis, however, suggests that HDL may compete with LDL for the same receptors, and, since it is removed from these receptors to the interior of the cell less rapidly than is LDL, it may block, or at least slow down, the rate of accumulation of cholesterol carried by LDL into the cell (10, 80). The degree of blocking may be regulated by the constitution of the HDL; for instance, it has been shown that a diet very rich in cholesterol produces a form of HDL which, although only a small fraction of the whole plasma HDL, becomes fixed to ASMC in vitro at an increased rate (47).

Centrifugal analysis of lipoprotein suggests some heterogeneity (44), three fractions being found in the LDL and three or four being found in the HDL; although it is possible that some are artifacts of centrifugation, others, at least, have distinct apoprotein structures. Modification of these structures (48) increases the diversity of their physiological actions, which suggests that activity may be determined more by apoprotein than by lipid structure. The diverse reactions of lipoprotein fractions on protozoa and the lack of specificity of action of the individual lipid components (see Trypanosomes) suggests that in this field also, diversity of action may be a function of apoprotein structure.

### Trypanosomes

Part of the life cycle of *T. brucei* occurs in the blood and involves the transformation of a long, narrow, and highly active trypomastigote, which emerges from the tissues, into a stumpy, leaf-

like, and rather sluggish form; during the course of this transformation, cell division occurs and the residual stumpy form, in which division has ceased, accumulates globules of lipid (61). At the same time, the stumpy form acquires an excess of cholesterol, mostly as the ester which is presumably contained in these globules (90). The stumpy forms are then destroyed, liberating their lipid, some of which is taken up by macrophages (M. Guy, unpublished data); the rest is probably mobilized (see Interactions of the Malaria Parasite). The destruction of parasites in the blood seems to be due to the liberation of intracellular hydrolytic enzymes which are secreted in response to the accumulation of lipid and begin to dissolve the cytoplasm of the cell; these events in the trypanosome (89) closely resemble those that occur in the ASMC of the rabbit aorta model (63).

The hydrolytic enzymes so far identified in trypanosomes undergoing dissolution are cathepsin D, acid phosphatase (89), and phospholipase A<sub>1</sub> (85). Phospholipase A<sub>1</sub> has been identified in both *Trypanosoma congolense* and *T. brucei* (87), and its action on phospholipids gives rise to a hemolytic mixture of free fatty acids, mainly unsaturated: C<sub>14:0</sub> (2.8%), C<sub>16:0</sub> (20%), C<sub>18:0</sub> (24%), C<sub>18:1</sub> (7%), C<sub>18:2</sub> (22.5%), and C<sub>18:3</sub> (2.8%); C<sub>18:2</sub> (linoleic acid) is said to be the most active as a hemolytic agent (85). Similar work on malaria parasites is described in Interactions of the Malaria Parasite.

The stages of *T. brucei* which occur in the blood do not synthesize sterols, and in this respect they differ markedly from mammalian cells; moreover, culture forms, which are presumably related to the stages found in the insect vector, the tsetse fly, do synthesize sterols, but these turn out to be phytosterols, such as ergosterol, and not, apparently, cholesterol (17). It seems likely, therefore, that the cholesterol and cholesterol esters accumulated in the stumpy trypanosomes are derived entirely from the blood and are therefore predominantly of dietary origin.

Free cholesterol and free fatty acids do not occur normally in the blood; they are incorporated into the lipoprotein, which is then absorbed by the trypanosome. Confirmatory evidence of this mechanism of uptake is provided by dietary experiments in which rats were fed on a diet deficient in fat; on this abnormal diet, an abnormal fatty acid (eicosatrienoic acid, C<sub>20:3n-9</sub>) appeared in the blood, replacing the normal C<sub>20:2</sub> and C<sub>20:3n-6</sub> acids. The abnormal fatty acid was, in these experiments, absorbed unchanged by the stumpy trypanosomes, and this provides additional evidence that absorption occurs via the lipoprotein, since in these experiments cholesterol and fatty acids were mainly found associated together with the phospholipids that

would be expected as normal constituents of plasma lipoprotein (90). Although it seems likely that cholesterol is carried into the trypanosome by LDL, as it is carried into the ASMC in humans, it is not possible to state this with any degree of assurance, since the experiments were carried out in rats. In rat blood, LDL is present in low concentration, so in this species HDL becomes the main carrier of cholesterol (79); however, accumulation of lipid occurs in the trypanosomes infecting a wide range of other species, including humans (57), so it is likely that the main carrier of cholesterol esters (be it HDL or LDL) is involved in the accumulation of cholesterol esters in the trypanosome, whatever its host species.

### LETHAL FACTORS IN PLASMA

#### Trypanocidal Activity of Plasma Against *Trypanosoma brucei*

It has been known for many years that a factor present in human plasma is lethal to *T. brucei* (41). This factor is also found in the bloods of higher apes and monkeys and seems to have been developed as a protective mechanism against trypanosomes. However, some strains of trypanosomes have become resistant to the trypanocidal factor, so that although humans are protected from most of the strains that infect cattle, antelopes, and carnivores, they remain susceptible to certain strains of *T. brucei* (which, to distinguish them from strains that do not infect humans, are usually placed in the subspecies *T. brucei gambiense* and *T. brucei rhodesiense*). The "gambian" and "rhodesian" strains of *T. brucei* are identical to the strains of *T. brucei* usually found in animals except in their ability to overcome the trypanocidal factor; these are the causative organisms of "African sleeping sickness" in humans (67). (Thomas and Breinl [84a] showed that *T. brucei gambiense*, inoculated intraperitoneally, could infect baboons, but gave a benign infection with very low parasitemia.) In contrast, the baboon, which has a particularly high level of trypanocidal activity in its plasma, cannot be infected with any strain of *T. brucei* unless the trypanosomes are injected directly into its cerebrospinal fluid, where the toxic lipoprotein factor occurs in low concentration (65). Early work appeared to suggest that the trypanocidal factor was related to antibody, although it was noted at the time that the plasmas of some individuals—particularly those who suffered from liver diseases, such as cirrhosis, infective hepatitis, or obstructive jaundice—had diminished trypanocidal activities (72). More recent work has also appeared to confirm the relationship of the toxic factor with antibody by associating its trypanocidal action with the macroglobulin fraction of plasma when this had been

fractionated by ion-exchange and gel filtration chromatography (34). However, it was also noted that lipoproteins were associated with the same chromatographic fraction of plasma, so the trypanocidal factor could not then be identified with certainty. The matter has not been settled conclusively by Rifkin (68, 69), who has shown not only that the trypanocidal activity is associated specifically with the HDL fraction but that its activity is greatly reduced in the plasma of a case of Tangier disease, a genetic abnormality in which HDL is virtually absent. However, Rifkin did not suggest that all of the HDL fraction was trypanocidal; indeed, she showed that the trypanocidal activity occurred in only part of the elution curve of HDL.

#### Possible Nature of Trypanocidal Factors

Two types of trypanocidal factor—active *in vitro* and *in vivo*, respectively—occur in the plasmas of humans and baboons, the proportions of these activities varying among different individuals; moreover, the stabilities of the two activities are not the same in any one plasma (32), and it is therefore likely that two substances are involved. Of these two, the *in vitro*-acting substance seems to be a fraction of HDL; the identity of the *in vivo*-acting substance is less certain, although Hawking et al. (34) suggest that it may be of a different nature, perhaps a substance which is self-activated in the recipient's body. One candidate for the role of activator or activated agent is the enzyme lecithin:cholesterol acyltransferase (EC 2.3.1.43), which is present in high concentrations in the bloods of humans, baboons, and other higher primates but is reduced in cases of liver disease (20), as are both trypanocidal factors. The action of lecithin:cholesterol acyltransferase is to incorporate cholesterol into lipoprotein, forming the cholesterol ester and displacing lecithin; HDL is most readily affected and can be changed in this way when the enzyme is transfused from one person to another (21). In addition, lipoprotein from one species can be transformed after transfusion into another species (7); it is therefore reasonable to suppose that the lecithin:cholesterol acyltransferase of one species might act on the lipoprotein of another and that this might occur in the *in vivo* test. One must, however, recognize that such evidence is indirect and that there is as yet no direct evidence in support of the hypothesis that the *in vivo* factor can be identified as lecithin:cholesterol acyltransferase.

#### Relationship Between Cholesterol Uptake and Trypanocidal Activity of High-Density Lipoprotein

What, then, is the relationship between the formation of lipid globules due to the uptake of

lipoprotein by the stumpy form of *T. brucei* and the trypanocidal activity of HDL? Are they the same phenomenon seen from different perspectives, are they closely related phenomena, or are they totally independent of one another?

The evidence so far seems to suggest that the formation of lipid globules and the action of trypanocidal HDL are not the same phenomenon, since the dynamics of the two processes differ considerably: the formation of globules is, on the one hand, a slow process which takes 3 to 5 days to reach a maximum, after which the trypanosomes are gradually destroyed over a period of about 24 h by liberation of hydrolytic enzymes, as described above (61); trypanocidal HDL, on the other hand, acts rapidly and will produce complete lysis on incubation *in vitro* for 15 min.

Not all strains of *T. brucei* accumulate globules of lipid in their cytoplasm. There is, indeed, great variability in the amounts of lipid taken up by different strains, ranging from strains found in southern Africa, where globules are large and the autolysis correspondingly great, through the more virulent strains found north of Lake Victoria, which accumulate only small globules, to strains without globules that have acquired an extra virulence by rapid passage between laboratory animals (57, 58). Although some strains visibly absorb substantial amounts of lipoprotein and others appear to absorb none, all strains are fully susceptible to the action of trypanocidal HDL unless they have acquired a specific resistance to it (see Trypanocidal Activity of Plasma Against *Trypanosoma brucei*). Even when such a specific resistance is present, only a few individual trypanosomes are able to resist the trypanocidal activity long enough to establish an infection; the majority of the inoculum is destroyed. It seems likely, although this cannot yet be proved, that the formation of lipid globules and the action of trypanocidal HDL are distinct yet related phenomena, but it is not yet clear in what way they are related.

#### Factors Lethal to Other Species of *Trypanosoma*

Although most work has been done on the effects of serum on *T. brucei*, other species of *Trypanosoma* are similarly affected; thus, the failure of other trypanosomes (such as *T. congolense* and *Trypanosoma vivax*) affecting cattle and game animals to infect humans may or may not be due to similar substances in human plasma. For instance, Hawking has shown that some strains of *T. congolense* and *T. vivax* may be highly resistant to human serum, indicating that the resistance of humans to these trypanosomes must be due to some other mechanism (31). Conversely, studies have been made on nonhuman host species that produce trypanocidal sub-

stances. One such species is the cotton rat, *Sigmodon hispidus*, which has in its serum a powerful trypanocidal substance lethal to *T. vivax* (83). The active substance is again found in the part of the chromatogram where lipoproteins and macroglobulins are eluted, and it was at first identified tentatively as a macroglobulin (83); the nature of the factor in cotton rat blood which kills *T. vivax* therefore should be reassessed.

### INHIBITORY FACTORS IN PLASMA

#### Action of "Supplements" on *T. brucei* and *Trypanosoma vivax*

Early studies on the lethal actions of human and baboon sera on *T. brucei* demonstrated that their trypanocidal actions could be inhibited by serum from another species of animal if it was given concurrently. Sheep or rabbit serum was usually, but by no means always, effective, whereas mouse serum was never effective (94). This work has not yet been repeated, but the focus of interest has shifted to the inhibitory effect of serum on the lethal factor (or factors) which kills *T. vivax* when it is inoculated into rats. *T. vivax* will not normally infect laboratory rats unless it is injected together with the serum of a susceptible animal, such as a cow, a sheep, or an antelope, and, although it is sometimes possible for a strain that has been passaged many times in the presence of supplements to "take" eventually without supplementation, repeated supplements normally have to be given in order to maintain the infection (14-16).

#### Action of "Supplements" on *Trypanosoma lewisi*

A similar phenomenon relates to the infection of mice with the rat trypanosome *Trypanosoma lewisi*. This infection will only occur if the mice are given rat serum supplements at intervals. The nature of the supplement has been studied in greater detail in the *T. lewisi*-mouse model than in the *T. vivax*-rat model, and it has been possible to exclude the participation of immunoglobulins M and G ( $\gamma$ -2). However, other potentially active substances remain in the fraction, and these include siderophilin, immunoglobulin G ( $\gamma$ -1) (27, 28). In these experiments the lipid content of the fraction was found to be below 0.5%, and although the possibility that a specific lipoprotein was present cannot be eliminated, it is clear that at so low a concentration it would have to be exceptionally active.

### LIPID DIETARY FACTORS ACTING ON PROTOZOA

#### Effect of Polyunsaturated Fat in the Diet on Parasitic Protozoa

Infections with a number of protozoal parasites are modified or even suppressed by

changes in the lipid concentration of the diet. Godfrey (22, 23) has shown that *T. congolense* infections are markedly depressed by feeding the host on a diet containing cod-liver oil. *T. vivax* infection maintained in rats with a sheep serum supplement was completely suppressed by cod-liver oil. No suppression was noted in infections with *Trypanosoma cruzi* or with *T. brucei*, although it is possible that the latter may have been one of the highly virulent strains (noted above) which do not visibly absorb lipid. *Babesia rodhani* and *Plasmodium berghei* were similarly inhibited by cod-liver oil. On further examination, Godfrey (24) and Taylor (82) were able to demonstrate that the toxic factors in cod-liver oil were the polyunsaturated fatty acids  $C_{20:4}$ ,  $C_{20:5}$ ,  $C_{22:5}$ , and  $C_{22:6}$ , of the family of linolenic acid ( $C_{18:3}$ ), to which they are degraded in vivo (36).

The action of these polyunsaturated fatty acids in inhibiting parasitemia could be prevented by increasing the dietary intake of  $\alpha$ -tocopherol (vitamin E) or other in vivo antioxidants which have the effect of preventing the formation of epoxides and free radicals that are formed from polyunsaturated fatty acids and are highly toxic to cells.

There has been some argument as to whether similar substances may be toxic to mammalian cells, and there is evidence that derivatives, possibly carcinogenic, of the peroxidized fatty acids may be stored in tissues when such acids are fed to mammals; however, no toxicity or storage occurs when dietary intake is also accompanied by an increase in  $\alpha$ -tocopherol, because presumably the peroxidized acids become reduced before intracellular absorption occurs (62, 64, 81). Free fatty acids as such do not normally occur in the blood; they are in fact highly toxic, especially the common saturated fatty acids (13, 35), but the extensive uptake of lipid by parasites, which Godfrey's experiments imply, is most likely to occur via the absorption of lipoproteins, and their activity, which is so much greater on the parasites than on the cells of the host, is probably due to preferential uptake of lipoprotein by the parasites.

#### Inhibitory Effect of Milk on Parasitic Protozoa

Mice fed on a diet of unextracted casein cannot support infection with *T. congolense*. This might be due to the presence either of lipid or of some other toxic factor, but is usually held to be due to the absence from milk of some dietary factor essential for the growth of *T. congolense* (37). A similar explanation is usually given for the inhibitory effect of a milk diet on *P. berghei* (30, 45) infection, in which the effect is usually assumed to be due to the absence of 4-aminobenzoic acid, an essential dietary factor

for the malaria parasite, which mammalian cells can mostly synthesize. However, in a number of experiments the expected inhibition did not occur (33), and it is possible that additional factors are operating. In one set of experiments (26), the inhibitory effect of a milk diet did not occur if skim milk was used, and this suggested that the presence of lipid might also be important and contribute to the inhibitory effects caused by the absence of 4-aminobenzoic acid; a similar experiment (74) was less conclusive in its result. Milk fats are 65% saturated ( $C_{14}$ ,  $C_{16}$ , and  $C_{18}$ ), but they also include a substantial (31%) amount of monounsaturated fats ( $C_{16:1}$  and  $C_{18:1}$ , mainly the latter); only a small amount of polyunsaturated (3.4%) linoleic acid ( $C_{18:2}$ ) is present (36). Since polyunsaturated fatty acid forms only a small proportion of the lipids of milk, it seems unlikely that the action of the lipid is due to oxidation or that it would be reversed by  $\alpha$ -tocopherol. Recent repetition of these experiments under more carefully controlled conditions (H. F. El Bashir, E. B. Fern, and W. E. Ormerod, unpublished data) showed that the inhibitory effect of skim milk was not primarily due to deficiency of lipid so much as to disturbance of appetite. Inhibition of malaria was thus caused by protein deficiency (18), and it is not related to the uptake of lipid.

#### DIRECT ABSORPTION OF LIPIDS BY PROTOZOA

##### Trypanosomes

Although free lipids do not normally occur in the blood, parasitic protozoa do apparently have the ability to absorb them. Thus, *T. lewisi* and *Trypanosoma equiperdum* (now usually considered to be a subspecies of *T. brucei*) have been reported to absorb globules of fat if these are added to the blood in which they are suspended (92, 93).

##### Leishmania

*Leishmania* species appear also to absorb micellar globules of lipid, although this process is probably more a function of the macrophage in which the amastigote of this parasite is harbored. The process has been used to present toxic chemotherapeutic substances to the parasite, since macrophage and parasite absorb the globules in greater concentration than do the other cells (4).

##### Entamoeba

*Entamoeba histolytica*, which is not a blood parasite, has the property of absorbing native cholesterol when this is added to the culture medium; under these conditions its behavior is

changed from that of a nonpathogenic species, which develops in the lumen of the gut without causing lesions, to a pathogenic species that invades the gut wall and produces ulcers (5, 76). Although this behavior appears to be well substantiated, it is in apparent conflict with the more recently accepted concept that the pathogenicity of *E. histolytica* is a genetically controlled factor that can be identified by isoenzyme markers (73); however, it is interesting to draw the analogy between the accumulation of cholesterol in *T. brucei*, with subsequent release of hydrolytic enzymes, and the possible requirement of cholesterol by *E. histolytica* in order that it shall produce the hydrolytic enzymes which are a necessary factor in its pathogenicity (55, 56).

#### INTERACTIONS OF THE MALARIA PARASITE

##### Absorption of Lipoprotein

Note has been taken above of the accumulation of lipid in the cells of African trypanosomes via the lipoprotein and of the trypanocidal action of HDL. It has also been noted that polyunsaturated fats and possibly other types of fat taken by mouth can inhibit the growth of malaria parasites. It is therefore pertinent to review evidence which might indicate that the malaria parasite also interacts with plasma lipoprotein in a manner similar to that of the trypanosomes.

Clofibrate, a synthetic drug which lowers the level of plasma lipoprotein, has been shown to decrease the parasitemia in experimental malaria caused by *P. berghei* (52); this activity suggests that lipoprotein may carry essential lipid metabolites which act as limiting factors on the growth of the malaria parasites, but the possibility that clofibrate acts directly on the parasite as an inhibitory agent cannot be excluded.

Experimental malaria (49–51) in mice appears to be associated with an increase in LDL and very low-density lipoprotein, whereas in humans infection with *P. vivax* has induced a condition resembling endogenous hyperlipemia (Frederickson type IV), a transient increase in LDL reversible by chloroquine therapy; however, in another study of patients infected with *P. vivax* (38), the opposite appears to obtain, with transient disappearance of plasma HDL and its reappearance after the parasitemia has been removed by chloroquine treatment. The authors of the latter study suggest that the HDL may have been taken up by the parasites and point to the very high concentrations of phosphatidylcholine in both the parasites and the HDL.

Extracts of *P. berghei* have been shown to contain sterol esters amounting to 65% of the

neutral fat lipids present, and, although the sterols involved were not identified in this instance, there is no evidence that any sterol other than cholesterol occurs in the malaria parasite (6). Since malaria parasites do not synthesize *de novo* either cholesterol or fatty acids (12), it seems likely that the sterol ester is derived entirely from absorbed plasma lipoprotein.

Although the evidence for absorption of lipoprotein by, and accumulation of cholesterol ester in, the malaria parasite is preliminary and somewhat inconclusive, it is reasonably consistent in supporting the hypothesis that the level of plasma lipoprotein is an important factor in the growth of the malaria parasite in the blood, but there are as yet no data on the mechanism of absorption, i.e., whether it occurs through the structure of the erythrocyte or directly from the plasma.

### Liberation of Hydrolytic Enzymes

The uptake of lipoprotein by the malaria parasite, as in trypanosomes, is followed by liberation of hydrolytic enzymes, and this suggests that a process similar to that described in Cholesterol Ester Accumulation for ASMC and trypanosomes also occurs in the malaria parasite. Evidence of a possible connection between absorption of lipid and the liberation of hydrolytic substances has arisen from the study of a so-called lytic factor isolated from several species of *Plasmodium*. This lytic factor, stated to lyse parasitized and normal corpuscles, has been said to consist of monounsaturated  $C_{18:1}$  fatty acids (39), and the main activity has been ascribed to vaccinic acid ( $C_{18:1w7}$ ), rather than to the more usual oleic acid ( $C_{18:1w9}$ ) (40). Recent work, however, has failed to identify vaccinic acid in isolates, but has disclosed a mixture similar to the hemolytic mixture of fatty acids obtained from trypanosomes (see Cholesterol Ester Accumulation) consisting of  $C_{16:0}$  (33%),  $C_{18:1}$  (36%), and  $C_{18:2}$  (18%) with the minor constituents  $C_{18:0}$ ,  $C_{18:3}$ , and  $C_{19:0}$  (together, 13%). Boiling for 5 min changes the proportion of these acids, and the lytic activity is reduced (6); although the authors state that vaccinic acid was not present, it is not clear from the methods described in the paper whether they were adequate to distinguish  $C_{18:1w7}$  from its isomer,  $C_{18:1w9}$ .

Although the mixture of fatty acids appears to have a specific hemolytic effect, whether produced from trypanosomes, malaria parasites, or from the cells of the host, it is important to emphasize that the liberation is likely to occur as a result of the production of hydrolytic enzymes by the parasite as it matures. Cathepsin D and acid phosphatase (as in trypanosomes) have been identified as being produced by several

species of malaria parasite (43) as they mature towards schizogony, and much of the activity remains in the erythrocyte ghost after the daughter parasites (merozoites) have been liberated into the blood. It is almost certain that lipids are liberated by a lipolytic enzyme similar to the phospholipase of trypanosomes. Such a process of liberating the lipid has been postulated by Maurois et al. (49).

### Further Metabolism of Liberated Lipid

Maurois and his colleagues further suggest that the enzymic liberation of lipid may give rise to an increased mobilization of lipid, which causes an increase in very low-density lipoprotein into which the lipids are rapidly incorporated; this process is followed, in turn, by a rise in LDL. Such a sequence of events suggests that the route of catabolism of lipids liberated by parasites in the blood may be similar to the route of those absorbed from the gut and mobilized according to the endogenous and exogenous cycles of Brown et al. (9).

Thus, an increase in lipoprotein in the blood may be caused by increased lipid mobilization by the parasite, but a decrease may occur as a result of the parasite consuming lipoprotein. The preliminary results of Lambrecht et al. (38) may be an example of this, but it is a difficult concept to establish because the increase in blood volume, which frequently occurs in pathogenic infections, would tend to cause an apparent fall in one factor or another.

### Similarities of Malaria and Trypanosome Infections

In malaria and trypanosome infections, a similar sequence of three events takes place: (i) absorption of considerable amounts of cholesterol esters of fatty acids, (ii) intracellular liberation of hydrolytic enzyme, and (iii) dissolution of the cell. The differences, however, are first that the trypanosome cell itself is destroyed, whereas the malarial merozoites escape from the dissolved erythrocyte, and second that it is only in the trypanosome that there is a clear causal relationship between the different stages of the lytic process; nevertheless, in both parasites the lytic process appears to be an integral part of the life cycle.

### CONCLUSIONS

#### Possible Evolutionary Relationship Between Aortic and Protozoal Disease

The object of this review has been to point out that there are analogies in the uptake of lipoprotein and the damage that it can cause to the cell that receives it, between, on the one hand,

mammalian body cells (notably, ASMC) and, on the other, various parasitic protozoa that are found in the blood. The study of lipid physiology in mammalian cells has, because of its importance to human arterial disease, become an advanced and sophisticated branch of science; similar studies on protozoa are in their infancy, but the diversity of effect, both in the direct action of lipoprotein factors which are toxic and in the indirect blocking action of others (or so the preliminary evidence seems to suggest), indicates that the study of lipoprotein-cell interaction in protozoa might illuminate significantly the study of mammalian lipid physiology (2).

Although it is not, perhaps, surprising that the study of parasites should reveal biochemical mechanisms that are similar to those of the host cells that they mimic, it is possible that the relationship between the actions of lipoprotein in mammals and in their protozoan parasites may be closer and more subtle than a mere similarity of biochemical mechanisms.

#### Hypothesis of Evolutionary Development of a Protective Lipoprotein

The overall function of lipoprotein is to carry essential lipid metabolites for the cells of the host, but it also carries lipid to blood parasites. Since parasites in the blood reproduce faster than the cells of the host, any effects, toxic or beneficial, that the lipoprotein produces are likely to be greater on the parasite than on the host. We suggest that in the course of evolution, modifications have occurred in the structures of certain lipoproteins in the blood that have made them toxic to parasitic protozoa. Such changes in structure are likely to give a net advantage to the host, but such an advantage may also be offset by certain disadvantages when the production of lipoprotein begins to assume a dual function, not only of transmitting lipid to body cells that require it but also acting as a protective mechanism. Through the period of evolution of the higher mammals, protozoa must have been important competitors and pathogens; but for modern humans, especially in the northern hemisphere where protozoal disease has largely been eradicated, their constraining influence has greatly diminished. Atherogenic diets, however, are a relatively modern phenomenon and are unlikely to have affected significantly the evolution of *Homo sapiens*.

Protozoal infection is an ancient phenomenon which may have antedated the evolution of vertebrates or even of an immune system as we know it today. Hosts, throughout the ages, have evolved a variety of strategies for ridding themselves of guests that have overstayed their welcome, and the specific lipoprotein factors which kill trypanosomes, and perhaps other parasites

as well, may be the survivors of some such primitive device. There may, indeed, be other diverse mechanisms which ensure a home only to the parasite that has specialized closely in colonizing a particular host species and which limit the aggression of other, merely opportunistic invaders. The highly specialized life cycles of the trypanosomes, malaria parasites, piroplasms, and other hematozoa are an indication of the extent to which mutual adaptation has occurred between parasite and host during the course of evolution.

The usefulness of possessing a lipoprotein factor which is lethal to an invading parasite has not been outdated in mammals by the possession of a fully developed system of humoral and cell-mediated immunity; the continued usefulness is shown in the higher primates by their possession of a highly effective trypanocidal factor in the HDL. Since trypanosomes are able to change their main antigenic structure with great speed (25, 70, 71), thereby evading the immune response, this additional protective mechanism is clearly of great value. Although sleeping sickness trypanosomes have in their turn been able to adapt themselves in such a way as to evade the lethal effects of HDL and establish an infection, this property should not obscure the fact that *Homo sapiens* and its near relatives continue to be protected from a range of trypanosome species widely prevalent in Africa with which the ruminants, which have no such effective factor, have had to come to terms by other means.

Humans, the higher apes, monkeys and, particularly, baboons have in their bloods the most effective trypanocidal factor possessed by any species, but they also seem to be the most susceptible species to the development of atheroma (1); the susceptibility of these species seems to support the hypothesis that the development of a factor toxic to trypanosomes also implies toxicity to the hosts' own cells. But the picture is a complicated one: there are at least two lipoprotein factors active against trypanosomes, a slow-acting factor (present in HDL in rats and possibly LDL in other species) and a fast-acting factor, particularly active in the HDL of humans and of baboons. There also appear to be inhibitory factors (usually referred to as serum supplements) in the bloods of a number of mammals; these substances may (as has been suggested above) also be located in the HDL. The inhibitory factors seem to act by competitive absorption into the trypanosome cell, thereby preventing the toxic factor (also probably in HDL) from being absorbed. The existence of toxic factors active against protozoa and inhibitory factors competing against them suggests an even greater degree of functional heterogeneity

of HDL than the interactions between mammalian cells and lipoprotein (discussed at the beginning of this review) seem to display, and the understanding of this heterogeneity in the study of protozoal lipid metabolism may contribute significantly to the study of mammalian physiology.

In mammalian cells, cholesterol ester is taken up mainly from the LDL, and this provides cholesterol and fatty acid additional to what is produced by intracellular synthesis, a process which is lacking in protozoal cells. It is generally believed that there is a homeostatic mechanism to balance absorption with synthesis, preventing excess absorption; when this mechanism breaks down, as it appears to do under the influence of certain toxic factors, excessive accumulation of cholesterol takes place. It is possible that one of these toxic processes may be the result of the same trypanocidal factor in the HDL which, having been developed as a defense against a foreign invader, may yet have some residual action against the host's own cells. Conversely, factors which inhibit the toxicity against human cells are believed also to occur in human HDL. Such factors appear to act by competing for the same loci on the cell that normally take up the LDL, and we suggest that they may be related to the inhibitory factors (serum supplements) which allow protozoal infection to occur in abnormal hosts. Excessive absorption or synthesis of lipoprotein might be removed from mammalian cells by a process of "cellular defecation" making use of the lysosome system. Although this process occurs in a wide variety of mammalian cells, the removal of excessive lipoprotein components has not been specifically identified; however, it does appear to occur in trypanosomes (89). Perhaps they need such a process more than do mammalian cells, since trypanosomes do not have any homeostatic mechanism to control the amount of lipoprotein that they absorb. If damage does occur, most mammalian cells can be replaced, like trypanosomes in blood; but this may not apply to cells which have a structural role, such as ASMC; it may be the irreplaceability of ASMC which makes them the point of least resistance in the host's defences against excessive lipid accumulation.

#### **Lack of Selective Pressure to Eliminate Toxic Factor**

It may be argued that had humans acquired a toxic factor which acted against their own cells, genetic pressure to reduce the prevalence of the allele would have increased as soon as subjection to parasitic protozoa had ceased to be an important feature of their environment; argu-

ments which suggest that such selective pressure is likely to be unimportant are as follows: first, humans have only recently (say, in the past century) lived in such a favored environment, relatively free from parasites and with an atherogenic diet available to them, and, second, although the mortality from atherosclerosis is high, it occurs mainly, if not exclusively, after the age of reproduction, and mortality from atherosclerosis is therefore unlikely to cause selective pressure. Although wild animals kept in captivity frequently develop atheroma (19), only the higher primates—together with the African elephant (77), which is so affected only under conditions of environmental stress—develop atheroma in the wild. During the period that humans or their forebears were developing antiprotozoal mechanisms, they were unlikely to have lived long enough or to have been so well-nourished as to have developed atheroma to the extent that this would have created any evolutionary disadvantage to outweigh the great advantage of possessing an effective antiprotozoal mechanism.

#### **Evolution of Parasitic Protozoa**

Not only have humans and their mammalian relatives undergone evolution, but so too have the protozoan parasites that infect them; in so doing they have even taken advantage of some of the protective mechanisms that the host has used against them, and the development of resistance in some strains of *T. brucei* to toxic HDL has been noted in Lethal Factors in Plasma. However, in addition *T. brucei* has evolved a life cycle in its mammalian host which actually requires the uptake of excess lipoprotein; one effect of this uptake is the accumulation of cholesterol esters, the intracellular liberation of hydrolytic enzymes, and the destruction of large numbers of circulating trypanosomes. One advantage of this process is in ensuring that the vascular system of the host does not become overloaded with vast numbers of trypanosomes and thereby killed; another is in producing the conditions described by Ormerod (59, 60) in which multiple division forms can be produced, for it is these forms, when they are intracellular, that provide optimal conditions for the survival of both host and parasite, the blood forms and other extracellular forms which endanger the host being merely the agents for the parasite's insect transmission. Malaria parasites may also have evolved to take advantage of the absorption of lipoprotein; we know that they accumulate cholesterol esters and that this is followed in sequence by intracellular secretion of hydrolytic enzymes and by destruction of the carrying cell. The sequence of events has a more obvious

causal connection in the trypanosome, but in the malaria parasite their causal connection remains hypothetical. Similarly, other protozoal parasites (*Babesia*, *Leishmania*, and *Entamoeba*) are known to absorb lipids, and some (*Entamoeba* and the trichomonads [54, 75]) are known to secrete hydrolytic enzymes. These hydrolytic enzymes have an importance in the pathogenesis of protozoal disease which is probably greatly underestimated; however, this aspect is not sufficiently close to the present topic to be pursued further in this review.

### Reasons for Pursuing Studies in Lipid Metabolism of Protozoa

Much more is known about the mechanisms of absorption of lipid into mammalian than about protozoal cell mechanisms; indeed, the lack of precise knowledge of the latter is often frustrating. However, it is possible that a more detailed knowledge of the lipid metabolism of protozoa might be of value in understanding mammalian mechanisms, and hence in understanding the pathogenesis of atheroma; for instance, if the inhibiting substances (serum supplements) which antagonize the ability of a particular host to resist a protozoal infection can be shown to be lipoprotein in nature, then the range of diversity of active lipoprotein factors, possibly active against mammalian cells, will have been extended and tools will have been provided for the study of LDL and HDL receptors and the ways in which they can be activated or blocked. The infant science of protozoal lipid metabolism may still have little to offer the wider field of mammalian lipid physiology in terms of concrete fact, but it does offer much in terms of illuminating hypothesis, usually the catalyst for further experimentation.

### SUMMARY

ASMC and sleeping sickness trypanosomes (*T. brucei*) both store globules of excess fatty acids esterified with cholesterol that are derived from the plasma lipoproteins; in both instances the globules are associated with intracellular liberation of hydrolytic enzymes that destroy the cells. The malaria parasite, *Plasmodium*, also absorbs lipoproteins, and hydrolytic enzymes are also liberated and the carrier cell (the erythrocyte) is destroyed, but connection between these processes (in *Plasmodium* and in several other blood protozoa) has not been established as clearly as in *T. brucei* and ASMC.

There is a diversity of serum factors—some lipoprotein, others as yet uncharacterized—which either assist the host in destroying its trypanosomal parasites or antagonize this action; analogous factors also affect cytolysis of

ASMC. Species with powerful trypanocidal factors seem particularly prone to develop atheroma. We suggest that lipoprotein factors have been evolved as antiprotozoal mechanisms but that they have residual toxicity against host cells, ASMC being particularly susceptible.

Genetic pressure on the parasite to adapt to such mechanisms has been strong, and populations of trypanosomes (e.g., *T. brucei rhodesiense*) have developed resistance to trypanocidal lipoprotein; however, genetic pressure on humans to eliminate alleles for toxic action against their ASMC is weak, because the ill effects of atheroma occur mainly after the age of reproduction and because atherogenic diets and environments free from protozoal pathogens are recent phenomena.

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